ECDYSONES AND SYNTHETIC ANALOGS: MOLTING HORMONE ACTIVITY AND INHIBITIVE EFFECTS ON INSECT GROWTH, METAMORPHOSIS AND REPRODUCTION

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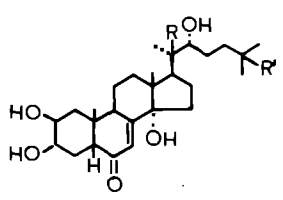
ABSTRACT

Five ecdysones and 19 ecdysone analogs were assessed for molting hormone activity in the house fly assay. When these 5β-steroids were further tested in the house fly, the confused flour beetle, the yellow fever mosquito, and the German cockroach, many of the compounds inhibited growth, development and/or reproduction. The relationship of structure to both the molting hormone and inhibitive activity of these compounds is discussed.

In previous studies (1, 2), we showed that a synthetic analog of the insect steroid molting hormones (ecdysones), when ingested, inhibited growth and metamorphosis and was also a female chemosterilant for certain insects. This report concerns the effect of structure on both the inhibitive effects and molting hormone activity of certain ecdysones and a number of synthetic ecdysone analogs and derivatives.

EXPERIMENTAL AND RESULTS

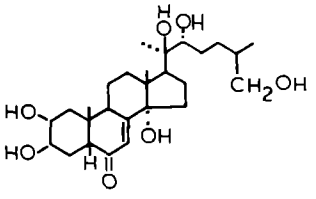
Compounds - The ecdysones tested (Fig 1) were either received as gifts, isolated from plants and/or insects, or purchased from a commercial source. Where necessary, the ecdysones were purified by adsorption chromatography and/or recrystallization. The purity of these compounds was assessed by melting point determination, thin-layer chromatography, and spectroscopic (UV, IR, NMR and Mass) analyses. The synthesis and properties of the synthetic ecdysone analogs (Fig 2) are reported in a companion paper in this issue of Steroids. Synthetic analogs I, V, and XVII were extracted from treated diets and analyzed by chromatography and spectroscopy to assess their stability. Less than 5% hydrolysis and/or decomposition was found to occur with these steroids.



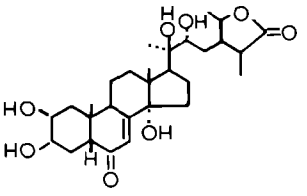
α-Ecdysone, R=H; R'=OH

20-Hydroxyecdysone, R=R'=OH

Ponasterone A, R=OH; R'=H



Inokosterone



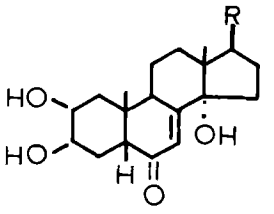
Cyasterone

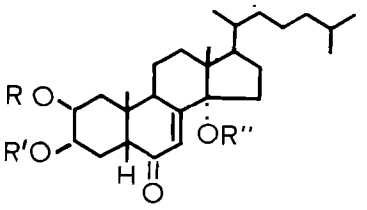
Fig. 1

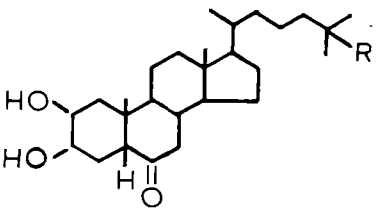
Molting hormone assay - Molting hormone (MH) activity was assessed by using the previously described house fly assay (3). House flies, Musca domestica Linnaeus, used in these assays and in all other house fly tests reported herein were from a 1948 NAIDM strain. Compounds were initially tested at 0.002, 0.005, 0.02, 0.05, 0.5, and 5.0 µg per ligated larva, either in water or, if not water soluble, in 5% Hydroxy Lecithin emulsion (4). The data in Table 1 report the lowest dose at which MH activity was detected (15-25% response), and the quantity required to give 50-60% response in the test insects (=1 house fly unit). The natural ecdysones tested were all more active than the synthetic analogs in the house fly assay.

The two major insect ecdysones (α-ecdysone and 20-hydroxyecdysone) and the phytoecdysone, ponasterone A, showed the highest MH activity and were about equally active. Only five of the synthetic analogs showed activity at the dosages tested; compound I, its 2β,3β-bis-(trimethylsiloxy) derivative (V), and its 2β-methoxy, and 3β-methoxy derivatives (IX, X) were 1/5 to 1/10 as active as the most active ecdysones, and the 6-hydroxy analog (XIX) of I showed slight MH activity.

House fly larval test - House fly larvae were reared aseptically on a semi-defined diet at 30° as previously described (5). The ecdysones and synthetic analogs were added to the diet by coating the compound on the dry dietary components with acetone or methanol. The relative biological activity of the compounds was evaluated by the percentage puparium formation ("pupation") and adult emergence based

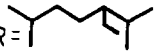






I, R = 

II, R = 

III, R = 

IV, R = 

V, R = R' = $(CH_3)_3$-Si; R'' = H

VI, R = R' = R'' = $(CH_3)_3$-Si

VII, R = R' = Ac; R'' = H

VIII, R = Ac; R' = R'' = H

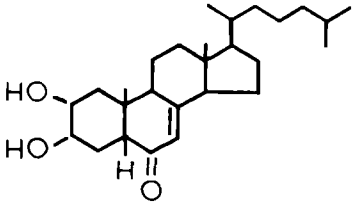
IX, R = CH_3; R' = R'' = H

X, R' = CH_3; R = R'' = H

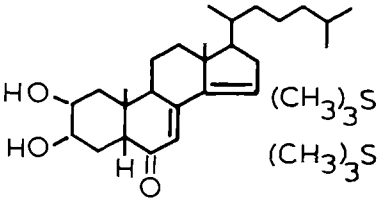
XI, R = R' = CH_3SO_2; R'' = H

XII, R = H

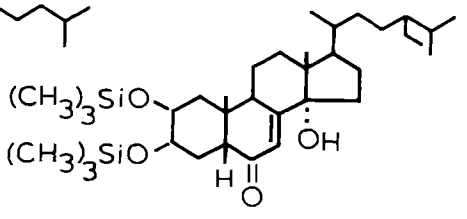
XIII, R = OH



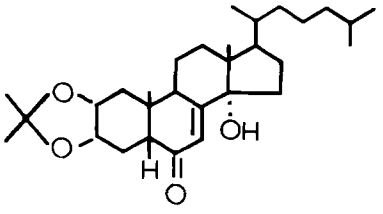
XIV



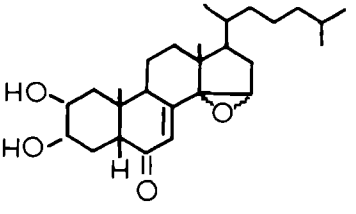
XV



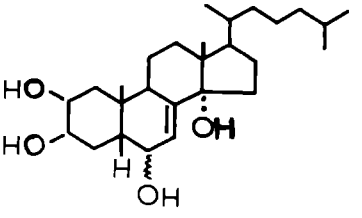
XVI



XVII



XVIII



XIX

Fig. 2

Key to Compounds in Figs. 1 and 2

α-Ecdysone = 2β,3β,14α,22R,25-Pentahydroxy-5β-cholest-7-en-6-one

20-Hydroxyecdysone = 2β,3β,14α,20,22R,25-Hexahydroxy-5β-cholest-7-en-6-one

Ponasterone A = 2β,3β,14α,20,22R-Pentahydroxy-5β-cholest-7-en-6-one

Inokosterone = 2β,3β,14α,20ξ,22ξ,26-Hexahydroxy-5β-cholest-7-en-6-one

Cyasterone = 2β,3β,14α,20ξ,22ξ,28-Hexahydroxy-6-oxo-5β-stigmast-7-en-26-oic acid γ-lactone

I = 2β,3β,14α-Trihydroxy-5β-cholest-7-en-6-one

II = 2β,3β,14α-Trihydroxy-(24R)-5β-ergost-7-en-6-one

III = 2β,3β,14α-Trihydroxy-(24R)-5β-stigmast-7-en-6-one

IV = 2β,3β,14α-Trihydroxy-27-nor-5β-cholest-7-en-6-one

V = 14α-Hydroxy-2β,3β-bis(trimethylsiloxy)-5β-cholest-7-en-6-one

VI = 2β,3β,14α-Tris(trimethylsiloxy)-5β-cholest-7-en-6-one

VII = 2β,3β,14α-Trihydroxy-5β-cholest-7-en-6-one 2,3-diacetate

VIII = 2β,3β,14α-Trihydroxy-5β-cholest-7-en-6-one 2-acetate

IX = 3β,14α-Dihydroxy-2β-methoxy-5β-cholest-7-en-6-one

X = 2β,14α-Dihydroxy-3β-methoxy-5β-cholest-7-en-6-one

XI = 2β,3β,14α-Trihydroxy-5β-cholest-7-en-6-one 2,3-dimethanesulfonate

XII = 2β,3β-Dihydroxy-5β-cholestan-6-one

XIII = 2β,3β,25-Trihydroxy-5β-cholestan-6-one

XIV = 2β,3β-Dihydroxy-5β-cholest-7-en-6-one

XV = 2β,3β-Dihydroxy-5β-cholesta-7,14-dien-6-one

XVI = 14α-Hydroxy-2β,3β-bis(trimethylsiloxy)-(24R)-stigmast-7-en-6-one

XVII = 2β,3β,14α-Trihydroxy-5β-cholest-7-en-6-one 2,3-acetonide

XVIII = 14ξ,15ξ-Epoxy-2β,3β-dihydroxy-5β-cholest-7-en-6-one

XIX = 5β-cholest-7-en-2β,3β,14α,6ξ-tetrol

Table 1 Molting hormone activity of ecdysones and analogs in the house fly assay.

Compound	Molting Hormone Activity*	
	Lowest Dose Detected (μg)	House Fly Unit (μg)
α-Ecdysone	0.002	0.005
20-Hydroxyecdysone	0.002	0.005
Ponasterone A	0.002	0.005
Inokosterone	0.005	0.01
Cyasterone	0.005	0.01
Compound I	0.01	0.03
V	0.01	0.03
IX	0.02	0.03
X	0.02	0.03
XIX	0.50	-

* All other compounds shown in Fig. 2 were inactive when tested at 5 μg/ligated larva.

on the number of viable eggs per flask. All compounds were initially tested at 150 μg/g (wet wt) of diet, and those compounds causing greater than 90% inhibition of adult emergence were retested at 75 and 15 μg/g.

Of the insects tested, house fly larvae were affected by the greatest number of the ecdysone analogs (Table 2). Only two of the natural ecdysones showed appreciable activity, and even the most active, ponasterone A, was considerably less inhibitory than a number of the synthetic analogs. The six most active analogs were retested at lower concentrations (Table 3), and the two most active, compound I and its 3β-methoxy derivative (X), reduced adult emergence by 60% at 15 μg/g of diet. The immature stages of the house fly differed from the other immature insects tested in that it was more susceptible to the ecdysones and analogs during the terminal (puparia-pupa-adult) molts than during the earlier larval molts.

House fly ovarian development test - Groups of unfed house flies (25 ♀ and 7 ♂) less than 6 hr old were anesthetized with CO_2 and transferred to half-pint cartons with screened tops. The cartons contained a watering device and 1 g of diet (6) consisting of sucrose, dry defatted milk, and dry whole egg (6:6:1). The ecdysones and analogs were coated on the diet at concentrations up to 0.5% (dry wt)

Table 2 Effects of ecdysones and synthetic analogs on puparium formation and adult emergence of house flies when added to the larval diet at 150 μg/g.

Compound	Percentage Inhibition of:*	
	Puparium Formation	Adult Emergence
Ponasterone A	38	80
Cyasterone	12	22
Compound I	95	100
II	15	62
IV	31	100
V	83	98
VI	12	61
IX	94	100
X	92	99
XI	4	17
XII	6	45
XIV	0	56
XV	9	20
XVII	40	92
XVIII	0	14

* Each test based on two or more replicates of 125-150 insects per 25 g (wet wt) of medium. Average control puparium formation and adult emergence was 90 and 80%, respectively. All other compounds shown in Figs. 1 and 2 caused less than 10% inhibition of adult emergence at 150 μg/g.

with acetone. The insects were held on the diet for 4 to 5 days at 28° and 50% RH at which time the females were sacrificed and their ovaries removed. The terminal oocytes of the ovarioles were measured, and the percentage of flies which showed severe inhibition of ovarian growth (length of terminal oocyte less than 0.3 mm) was recorded.

All five of the ecdysones and five of the synthetic analogs severely inhibited ovarian development at dietary concentrations of 0.5% or less (Table 4). The two most active compounds in this test system were the phytoecdysone cyasterone and the synthetic steroid compound I, which were about equally inhibitory. In addition to blocking the normal growth and maturation of terminal oocytes, the test steroids caused a number of morphological abnormalities in developing or developed oocytes. These included "tear-drop" shaped oocytes or oocytes with blunt ends often incompletely filled with yolk.

Table 3 Effects of the most active synthetic ecdysone analogs on puparium formation and emergence of house flies.

Compound		Amount in Larval Diet (μg/g)	Percentage Inhibition of: Puparium Formation	Adult Emergence
Compound	I	75	71	96
		15	8	60
	IV	75	22	74
		15	10	17
	V	75	73	90
		15	15	20
	IX	75	76	95
		15	15	20
	X	75	72	86
		15	53	60
	XVII	75	27	75
		15	9	9

Also observed were a reduced number of developed oocytes per ovariole and ovarioles containing oocytes at all different stages of development (asynchrony) including, in some cases, oocytes that were seemingly being resorbed.

House fly reproduction test - Unfed house flies (75 ♀ and 30 ♂) less that 18 hr old were placed in screen cages containing water and 10 g of a diet composed of sucrose, dry defatted milk and dry whole egg (42.5:42.5:15). The ecdysone analogs which were inactive in the house fly ovarian development test at 0.5% and compound I were coated on the diet at 0.1% (dry wt) with acetone. The flies were held at 28° and 50% RH and on the sixth and twelfth day after being placed on the diet, eggs were collected. From each cage, 4 aliquots of approximately 100 eggs were used to determine hatch, and 2 groups of 250 eggs were placed on CSMA medium to determine any effects on larval development, puparium formation, and adult emergence. The volume of the remaining eggs was taken to measure the total egg production (1 ml = approximately 10,000 eggs). The biological activity was assessed by comparing the average percent egg hatch, the total egg production, and the viable egg production of the flies held on treated diets with that of the controls.

Table 4 Effect of ecdysones and synthetic analogs on ovarian development in the house fly.

Compound	Percentage Ovarian Inhibition*			
	Concentration in Diet (%)			
	0.05	0.10	0.25	0.50
α-Ecdysone	-	14	50	100
20-Hydroxyecdysone	-	0	0	46
Ponasterone A	-	10	41	80
Inokosterone	-	0	13	56
Cyasterone	20	74	92	96
Compound I	23	70	92	100
IV	0	13	63	92
V	0	41	60	96
IX	0	26	68	96
X	0	25	46	64

* All other compounds shown in Fig 2 caused less than 10% inhibition at 0.5%.

Only four of the synthetic analogs (II, XIV, XVII, XIX) which were not inhibitors of house fly ovarian growth at a dietary concentration of 0.5% caused an appreciable reduction in progeny at 0.1%, and these are compared with compound I in Table 5. These four compounds were also found to cause a high percentage of abnormal developing or developed oocytes in the ovarian development test. A slight additional reduction in the number of progeny forming puparia and adults was also found to occur with all the synthetic steroids listed in Table 5. When males and females were fed separately on I, only the female reproductive system was affected.

Confused flour beetle larval test - Newly hatched confused flour beetle larvae, Tribolium confusum Jaquelin du Val, from a laboratory colony were reared to adults at 30°C and 55% RH on diets containing the ecdysones and synthetic analogs. The diet was composed of whole wheat flour, white flour, and dried brewers yeast (4:4:1) which had been autoclaved, pulverized with a hammer mill, and passed through a 60-mesh screen. The ecdysones and synthetic analogs were coated on the diet with acetone or methanol. The compounds were initially tested at 0.5% (dry wt) and those showing inhibitive effects were tested at 0.05%. The test system consisted of either 100 larvae per 2.5 g or 40 larvae per g of diet, depending upon the availability of the compound. The inhibitive effect was determined from the number of larvae developing to normal adults as compared with controls.

Table 5 Effect of synthetic ecdysone analogs on house fly reproduction when fed in the adult diet at 0.1%.

Compound		Percentage Inhibition of Egg Production*		Percentage Egg Hatch
		Total Eggs	Viable Eggs	
Compound	I	82	88	61
	II	14	25	80
	XIV	46	53	77
	XVII	50	63	81
	XIX	13	25	79

* Based on mean control values of 13,700 total eggs and 12,100 viable eggs (88.3% hatch). All other compounds shown in Fig 2 which were inactive in the ovarian development assay when tested at 0.5% were also inactive in this test at 0.1%.

Tribolium larvae were least susceptible to the ecdysones and analogs of the immature insects studied. The most active steroid for this insect was the phytoecdysone ponasterone A, and two of the analogs, I and IV, showed comparable activity (Table 6). The steroids exerted their effect primarily during the larval molts which resulted in growth retardation and mortality during the molting process as evidenced by dead larvae with the exuvia still attached. The compounds had a lesser effect on the pupa to adult transformation.

Confused flour beetle reproduction test - Groups of newly emerged adult beetles (25 ♀ and 25 ♂) were placed in a one-oz glass jar containing 1 g of the previously described Tribolium diet. The test compounds were coated on the diet at 1.0% by using acetone or methanol as the solvent. After 10 days on the treated diet, the insects were transferred at weekly intervals, for 4 weeks, to 20 g of untreated diet. Throughout the test period, the insects were held at 30°C and 55% RH. The number of progeny was recorded for each of the one-week periods, and the percentage inhibition was determined by comparison with controls.

Only one of the ecdysones, 20-hydroxyecdysone, appreciably inhibited reproduction in Tribolium at 1.0% (Table 7); another, ponasterone A, caused about 45% mortality at the test concentration, but the survivors produced the same relative number of progeny as the controls. One of the synthetic steroids (IV) also caused mortality and severely inhibited reproduction in the surviving insects. Three other synthetic compounds (I, VII, X) produced greater than 90% inhibition with no significant reversal of the inhibitive effects over the 4-week period. Compound I was tested at lower concentrations,

Table 6 Effect of ecdysones and synthetic analogs on development of the confused flour beetle.

Compound	Percentage Inhibition of Development*	
	Concentration in Larval Diet (%)	
	0.50	0.05
α-Ecdysone	28	0
Ponasterone A	100	75
Cyasterone	16	0
Compound I	100	32
IV	100	43
V	70	16
VII	29	0
VIII	12	0
IX	69	0
X	40	0
XIV	27	0

* Based on number of insects developing to adults; all other compounds listed in Figs. 1 and 2 caused less than 10% inhibition at 0.5%.

and in another experiment, the test period was extended to 8 weeks. This analog caused a 70% reduction of progeny at 0.5% but was inactive at 0.1%. No recovery in the reproductive capacity was observed, even at the end of 8 weeks with a dietary concentration of 1.0%. As with the house fly, only the female reproductive system was affected in Tribolium.

Yellow fever mosquito larval test - Twenty newly hatched yellow fever mosquito larvae, Aedes aegypti (L.), were transferred into 100 ml of distilled water in a 150 ml beaker. The ecdysones and synthetic analogs were added to the water in 0.05 to 0.2 ml of distilled acetone with thorough mixing at least 2 hr prior to adding the larvae. The compounds were initially tested at 1.0 and 0.1 ppm, and those that gave 90% or greater inhibition at 0.1 ppm were also tested at 0.01 ppm. The larvae were fed on micropulverized dog food (Gaines Dog Meal) and reared through to maturity at 25°C. The inhibitory effect of the compounds on development was calculated from the number of treated larvae forming pupae and adults as compared with controls.

Although the yellow fever mosquito larva was moderately susceptible to only one of the ecdysones, development in this insect was

Table 7 Effect of ecdysones and synthetic analogs on reproduction of the confused flour beetle when fed in the adult diet at 1.0% for 10 days.

Compound	Percentage*	
	Inhibition	Recovery
20-Hydroxyecdysone	70	25
Compound I	98	2
IV**	95	4
V	49	24
VII	98	1
VIII	77	11
IX	91	14
X	92	7
XIX	60	12

* Percentage inhibition is based on the average for 4 one-week egging periods; percentage recovery is the difference in inhibition in the 1st and 4th egging periods. All other compounds shown in Figs. 1 and 2 caused less than 10% inhibition at 1.0%.

** Toxic

severely inhibited by a number of the analogs at concentrations of less than 1 ppm (Table 8). Compound I was tested at concentrations intermediate between 0.1 and 0.01 ppm, and 25 ppb was found to inhibit larval development in about 90% of the insects. As with confused flour beetle larvae, the inhibitory effect with mosquito larvae was observed to occur primarily during the early larval molts and resulted in retarded growth and mortality.

German cockroach nymphal test - Twenty newly hatched German cockroach nymphs, Blattella germanica (Linnaeus), were transferred into a screened top half-pint jar. The jars contained a watering device consisting of a shell vial filled with 3% agar gel and diet consisting of a mixture of dog food (micropulverized), dried brewers yeast (USP), Liver Fraction L, and glucose (80:10:5:5) (7). The insects were held at 25°C and 50% RH. The ecdysones or synthetic analogs were coated on the diet with acetone or methanol. The compounds were tested at 0.5 and 0.05% (dry wt), and the inhibitive effect was determined by the number of nymphs developing to normal adults as compared with controls.

Table 8 Effect of ecdysones and synthetic analogs on development of the yellow fever mosquito.

Compound	Percentage Inhibition of Development*		
	Concentration in Water (ppm)		
	1.0	0.10	0.01
Ponasterone A	60	0	-
Compound I	100	100	15
IV	100	15	-
V	100	100	0
IX	100	100	15
X	100	90	0

* Based on number of insects developing to adults; all other compounds listed in Figs. 1 and 2 caused less than 10% inhibition at 1 ppm.

Table 9 Effect of ecdysones and synthetic analogs on development of the German cockroach.

Compound	Percentage Inhibition of Development*	
	Concentration in Diet (%)	
	0.50	0.05
Compound I	100	100
II	70	0
IV	100	32
V	100	100
VIII	100	0
IX	100	40
X	100	100
XIV	100	0
XVII	100	0
XIX	100	0

*Based on number of insects developing to adults; all other compounds shown in Figs. 1 and 2 caused less than 10% inhibition at 0.5%.

German cockroach nymphs were somewhat more susceptible to the ecdysone analogs than confused flour beetle larvae but were unaffected by any of the natural ecdysones at the highest concentration tested (Table 9). The steroids which caused complete inhibition of development at 0.05% (I, V, X) were all inactive at 0.005%. Compound I was tested at intermeditate concentrations between 0.05 and 0.005%, and 0.01% was found to cause appreciable growth inhibition. The nymphs appeared to be affected primarily at the time of molt, and dead nymphs usually had the exuvia still attached.

DISCUSSION

The five natural ecdysones tested differed only by a factor of two in MH activity in the house fly assay, but all of these compounds were considerably more active than the most active of the synthetic analogs. Compound I, which possesses the ecdysone nucleus and the cholesterol side chain, and three of its derivatives had the highest MH activity of the analogs. Except for cyasterone, compound I differs in structure from the natural ecdysones tested only in the absence of hydroxyl groups on the side chain. The presence of hydroxyls at specific positions in the side chain must then be a prerequisite for maximum MH activity. An additional methyl (II) or ethyl (III) at C-24 on the side chain of I or removal of a terminal methyl group (IV) results in the complete loss of MH activity in the house fly assay. The inactivity of the two phytosterol analogs (II, III) is in keeping with and indeed confirms prior nutritional and biochemical data on the essential cholesterol requirement of the house fly, an insect that is unable to dealkylate phytosterols (8, 9).

The three derivatives of I that showed MH activity involved substituents at the 2β- and/or 3β-hydroxyl groups; the 2β- or 3β-methoxy (IX, X), and the 2β,3β-bis-(trimethylsiloxy) derivatives (V). However,

other substituent groups at these positions of I such as acetoxys (VII, VIII), methanesulfonates (XI), or an acetonide (XVII) resulted in a complete loss of MH activity.

The 14α-hydroxyl group was also found to be essential for MH activity of I as evidenced by the inactivity of XIV, and in this respect the house fly differs from Calliphora (10). Introducing a double bond at the C-14 position of XIV to form XV or an epoxy group at C-14,15 (XVII) resulted in compounds that were also inactive. The importance of the 14α-hydroxyl group is further substantiated by the finding that substituting a trimethylsiloxy group for the 14α-hydroxy in compound V completely eliminates the MH activity of the latter compound. The low level of activity observed with the 6-hydroxy analog (XIX) of I suggests that a small percentage of this compound is oxidized in vivo to I in the ligated house fly larva.

Whereas the ecdysones were more active than the analogs in the MH assay, the synthetic steroids, when ingested, generally were more potent inhibitors of insect growth, metamorphosis, and reproduction. However, two of the phytoecdysones provided exceptions to this general trend: cyasterone was as active as the most active synthetic analog (I) in the house fly ovarian development test, and ponasterone A was the most active inhibitor in the confused flour beetle larval test. One of the major insect ecdysones, 20-hydroxyecdysone, showed either low inhibitive activity or was inactive in all the test systems except the confused flour beetle reproduction test where it was the most active of the ecdysones. Interestingly, the hemimetabolous

German cockroach was not affected by any of the natural ecdysones at the concentrations tested.

Five of the synthetic analogs, I, IV, V, IX, and X, were the most active inhibitors in all the development and reproduction test systems, except that V was only moderately active in the confused flour beetle reproduction test. These compounds, with the exception of IV, were also the most active analogs in the molting hormone assay. This taken with the observed effects on molting and morphogenesis supports our previous premise (1) that the inhibitive action of these steroids is in part related to their hormonal activity. We have previously noted (1) that certain of the analogs, particularily I, could well be intermediates in the biosynthesis of the ecdysones. Indeed we have recently shown that when ^{3}H-labeled I is injected into diapausing tobacco hornworm pupae, Manduca sexta (Johannson), diapause is terminated, and I is efficiently converted into the insects' two major ecdysones, α-ecdysone and 20-hydroxyecdysone (11). Such steroids as I then could exert their higher inhibitive activity through a more ready absorption (2) and a slow conversion to the insects' ecdysones, thus maintaining hormone titers in the insect that interfere with normal molting and metamorphosis. The high inhibitive activity of IV and its total lack of molting hormone activity is not currently understood, but one explanation is that this compound may be an anti-hormone or a hormone antagonist, and this possibility is currently being tested.

A further comparison of structure vs. inhibitive activity reveals

some interesting similarities and differences to those relationships found for MH activity. A methyl group at C-24 (II) resulted in the loss of inhibitory activity in all tests except the house fly larval and reproduction test and the German cockroach nymphal test and in these two immature test insects II was only about 1/10 as active as I. The addition of an ethyl group C-24 (III) completely eliminated inhibitive activity in all systems, just as it eliminated MH activity in the house fly assay. The 26-nor homolog of I, as mentioned, was highly inhibitive in all the development and reproduction tests, even though it was without MH activity in the house fly assay.

The 14α-hydroxy group was found to be important but not essential to inhibitory activity in all the test insects. Removal of this hydroxyl group from I resulted in a compound (XIV) without detectable activity in the house fly ovarian development and confused flour beetle reproduction test, but which was approximately 1/10 as active as I for house fly and Tribolium larvae and German cockroach nymphs. The analogs of XIV with substituents at C-14 were active only in the house fly larval test, with either a double bond (XV) or an epoxy group (XVIII) resulting in a reduction in activity. The removal of the double bond in XIV, to form XII, completely eliminates activity in all test systems except house fly larvae, in which the two compounds were about equally effective.

A number of examples of selectivity were also observed. The acetonide of I (XVII) was quite active in the house fly larval and cockroach nymphal tests and severely affected reproduction in the

house fly although it did not effectively block ovarian development. This latter observation and similar results with 3 additional analogs reemphasizes that although the ovarian development assay is a simple and rapid indicator of female chemosterilant activity in insects, the final criterion for evaluating the effect of a compound on reproduction must always be its effect on the production of viable progeny. The 2β,3β-diacetoxy (VII) and the 2β-acetoxy (VIII) derivatives of I were slightly active for Tribolium larvae but were quite effective against the adult insect. The diacetate (VII) was the more active of the two in Tribolium but only the monoacetate was inhibitory for German cockroach nymphs. The 6-hydroxy analog (XIX) that had slight MH activity was also active in the house fly and Tribolium reproduction tests as well as in the cockroach nymphal test.

One structural feature which we previously reported to be essential for both molting hormone and inhibitive activity is the 5β-H (A/B ring cis) (1,2). This was further confirmed in the present study by feeding the 5α-analogs of a number of the more active synthetic steroids. Concentrations of the 5α-analogs equal to or greater than that required to give complete inhibition with the 5β-analog were found to be inactive. Thus, we are at loss, even taking into account the different routes of administration, to explain the previous reports that 5α-6-keto steroids cause molting deficiences in Pyrrhocoris apterus (L.) (12,13) and have a sterilizing effect on the female house fly (14). The 5β-H structure is also essential for the female chemosterilant activity of ecdysone analogs in the boll weevil, Anthonomus grandis, Boheman (15).

The results reported herein and from previous studies with the ecdysones and analogs (1,2,15,16) indicate that there may be a wide variation in the susceptibility of different insects to these compounds and an equally wide variation in the response of a single species to different molecular structures. Certain of these steroids block insect growth and development at concentrations in the ppm to ppb range and inhibit ovarian development indicating their potential as hormonal pesticides and chemosterilants. Important advantages have been visualized for the use of insect hormonal chemicals in insect control in lieu of certain of the pesticides in current usage, and at the present state of development the juvenile hormone (JH) chemicals seem to offer the greatest immediate potential. The most active JH compounds are relatively simple molecules which lend themselves to commercial production; these compounds also readily penetrate the insect cuticle, many are good fumigants, and the most active of these chemicals are effective in the picogram range (17). Although some are female chemosterilants and certain others have ovicidal activity, the major effect (morphogenetic) of JH compounds on immature insects is to selectively interfere with the terminal molt; the pupal-adult or nymphal-adult molt.

Compounds with MH activity, however, because of their complex steroidal structures, would be more expensive to produce. These steroids do not readily penetrate the insect cuticle and thus must usually be taken up by ingestion. These disadvantages, however, may eventually be overcome through the development of active compounds with simpler structures, microbiological syntheses and/or by obtaining these steroids from plant sources. Already ecdysone analogs and derivatives have been prepared which penetrate the cuticle of certain

insects (2). The ecdysones and analogs have a decided advantage over the JH compounds in that they block immature development at any larval or nymphal molt and thus could be fitted directly into our present insect control technology. Many of the MH compounds are female insect chemosterilants and the effect on reproduction is often irreversible (2,15). Certain of the ecdysone analogs have the added advantage of being readily synergized so that their effect on either growth and development and/or reproduction may be enhanced as much as 10- to 20-fold (2).

The ecdysones control the molting and morphogenesis of arthropods other than insects including crustaceans and arachnids (18). There is also evidence that the growth, development, and reproduction of certain other of the polymorphic invertebrates is controlled by ecdysone or ecdysone-like steroids. Cleveland (19) has shown that the ecdysone of the insect host controls the sexual cycle of the symbiotic flagellate protozoans which inhabit the intestinal tract of the wood-eating roach Cryptocercus punctulatus Scudder. High sustained titers of ecdysones were found to bring about aberrations or disruption of the sexual cycle of these protozoans (20). More recently Muftic (21) has isolated from a mollusk, the snail Australorbis glabratus, a substance with properties similar to the ecdysones and which exhibits molting hormone activity in the house fly assay. This snail serves as an intermediate host for Schistosoma mansoni and the isolated substance was found to promote the in vitro metamorphosis of this trematode from the miracidial stage to the infective cercarial stage. The ecdysones and related steroids then may also find use as chemotherapeutic agents for protozoan or metazoan parasites of man and domestic animals, particularly those

such as the causative agent of malaria, which undergo a complex developmental cycle in both a vertebrate and an invertebrate host.

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